REMARKS

Claims 1-17 are pending in the present application with claims 16-17 being withdrawn by the Examiner as being directed to a non-elected invention. Applicants acknowledge and appreciate that claim 12 has been considered along with claims 1-11 and 13-15.

Claims 1, 3, 7-9, 11 and 13 are amended as indicated above, and claim 14 is canceled. In particular, independent claims 1 and 13 now provide a positive recitation that the methods are conducted in an atmosphere having less than 5 ppb ozone, and further reciting that the atmosphere is one that produces less than 10% degradation of the nucleic acids over a period of 1 hour at a temperature of from 18°C to 25°C. Claims 7-9 are amended to provide the recitation that the substrate is a planar support (e.g., a chip or wafer as is commonly used in light-directed synthesis).

Support for the requested amendments can be found in the specification at, for example, page 16, lines 4-20 (for ozone concentrations and degradation percentages) and page 13, lines 25-27.

Applicants believe no new matter is presented in any portion of the present amendment.

Rejection of Claims 1-15 Under 35 USC § 112, Second Paragraph

Claims 1-15 stand rejected under 35 USC § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

A. In view of the amendment to Claim 1, Applicants believe this aspect of the rejection is overcome. Additionally, Applicants note the term "degradation" is used in the specification along with the definition of "non-oxidizing atmosphere" to

refer to an process which would result in a given nucleic acid or probe appearing to be different from the undegraded nucleic acid or probe by HPLC (see page 6, lines 16-29).

B. through E. The term "non-oxidizing atmosphere" is meant to refer to the gas-phase atmosphere as well as the reagents in solution form. As the Examiner is aware, most reactions in an organic chemistry lab are run in solvent, many of them either open to the atmosphere (air) or under an atmosphere of nitrogen or argon. Once the reaction is completed, the reaction mixture is worked up using various wash steps, drying and removal of solvent. Accordingly, the present use of a "non-oxidizing atmosphere" is meant to refer to the chemical steps taking place on the solid support in which reagents are introduced to the support, but under an atmosphere that does not oxidize the product or products being formed. Reconsideration of this aspect of the rejection is respectfully requested.

F. Claims 7 and 9, while appearing to be similar, are meant to encompass those methods in which a light source is used to irradiate the substrate from the backside (relative to the nascent nucleic acid formation, see Claim 7) and in which the light source may be from any position, but is focused and directed to the backside using, for example, mirrors (see Claim 9).

G. In view of the amendment to Claim 11, Applicants respectfully request that this portion of the rejection be withdrawn.

H. Claim 13 is meant to include those embodiments in which an array of nucleid acids is prepared by attaching a plurality of pre-formed oligonucleotides to a support in a non-oxidizing atmosphere as newly amended.

In view of the above, Applicants respectfully request that each of the noted aspects of the rejection be withdrawn.

Rejection of Claims 1, 2, 3, 8, 13 and 15 Under 35 USC § 102(b) ("Terrett")

Claims 1, 2, 3, 8, 13 and 15 stand rejected under 35 USC § 102(b) as allegedly being anticipated by Terrett, N.K., "Combinatorial Chemistry" (1998) Oxford University Press, pp. 40-47 ("Terrett"). Applicants respond in part by amendment and in part traverse.

The Examiner has noted that Terrett describes various methods of synthesizing oligonucleotide arrays on a solid support (see Paper No. 5, page 5, section (2)). The Examiner further notes that Terrett describes an octanucleotide array, produced in 4 hours by activation of a support, attaching nucleotides to the support in defined regions that are not masked, using protecting groups and cycles of nucleotide additions to prepare the array. The Examiner further notes that the library of oligonucleotides was successfully used in hybridization studies, indicating that no substantial degradation had occurred. Applicants respectfully disagree with this conclusion, and with the Examiner's further notation that "[o]ne may conclude from the results of the hybridization assay that the library was synthesized in a `non-oxidizing atmosphere' and that the reference anticipates claims 1, 2, and 13." (Paper No. 5, *ibid.*).

In particular, following the pioneering work of scientists at Affymetrix in polymer array preparation, more specifically, oligonucleotide array preparation (see, for example, U.S. Patent No. 5,143,854, the parent application to the continuation-in-part application that matured into U.S. Patent No. 5,424,186, cited by the Examiner), a significant market developed for such arrays. It is clear that these arrays were quite useful for their intended purpose or such markets would hardly have developed. As a result, Applicants do not take issue with the disclosure of Terrett, but believe the conclusions set out by the Examiner (that the art describes arrays that work for their intended purpose and must therefore have been made by Applicants' process) are erroneous. Applicants respectfully note that improvements in technology continue to be the focus of ongoing research. One such improvement is the subject of the present application.

In particular, due to the sensitivity of instruments for oligonucleotide hybridization detection (e.g., using fluorescently labeled oligonucleotides), it is possible to detect hybridization even though there has been substantial degradation. Nevertheless, one can make significant improvements in arrays by reducing the amount of degradation that occurs and thereby increase the signal that is detected by the instrument.

In the present invention, methods of preparing nucleic acid arrays are provided in which the synthesis steps are carried out in a non-oxidizing atmosphere containing less than 5 ppb ozone such that less than about 10% degradation of the nucleic acid occurs. Terrett fails to disclose or suggest any method in which the reactions are run in an atmosphere containing less than 5 ppb ozone such that less than about 10% degradation of the nucleic acid occurs.

The Examiner has further relied on Fodor, et al., U.S. Patent No. 5,424,186 as inherently disclosing the light-directed synthesis of oligonucleotides under the non-oxidizing conditions specifically recited in Applicants' Claim 3.

Applicants' respectfully note that the reasoning underlying this aspect of the rejection is flawed. In particular, the Examiner points to example 3 of Fodor, as an indication that poly dT bound to a solid support was able to hybridize to poly dA probes even after an overnight incubation in 1X SSPE buffer at 40°C. The Examiner concludes that the poly dT probe must not have degraded. Once again, the ability to detect hybridization is *not* an indication of degradation, whether it be 1%, 5%, 10%, 40% or 95%. The ability of poly dT to hybridize to poly dA (wherein many copies of the oligonucleotides are present) is simply an indication of whether there exists sufficient poly dT for detection to take place. Depending on the amounts of probe made, as much as 95-99% can be degraded, yet hybridization might still be detected.

Applicants have demonstrated that by carefully excluding ozone from the procedures involved in nucleic acid array preparation, as well as packaging, arrays can be prepared in which degradation of the nucleic acids has been minimized, leading to improved and more sensitive arrays.

The Examiner has set forth the above rejection of claims 1, 2, 3, 8, 13 and 15 based on flawed assumptions of what must be inherently disclosed in the cited art.

Applicants respectfully request reconsideration in view of the specific limitations now incorporated in claim 1 and the remarks above.

Rejection of Claims 1, 2, 3, 10 and 13 Under 35 USC § 102(b,e) ("Fodor")

Claims 1, 2, 3, 10 and 13 stand rejected under 35 USC § 102(b) as allegedly being anticipated by Fodor, et al., U.S. Patent No. 5,424,186. Applicants respectfully traverse.

Fodor, et al. is cited as disclosing a method for synthesizing oligonucleotides on a solid support. Applicants' agree. The Examiner then concludes:

> the method Fodor et al. is performed in a `non-oxidizing atmosphere' in accordance with applicant's definition as evidenced by column 50, lines 23-37 in which Fodor et al. refers to the selection of protecting groups `*they are stable (that is , they remain attached to the molecule) to the synthesis reaction conditions; they are removable under conditions that do not adversely affect the remaining structure etc.* (See Paper No. 5, page 6, section 3., emphasis in original)

Here, the Examiner has apparently concluded that the use of certain reagents for the removal of protecting groups must mean the entire synthesis is carried out in a non-oxidizing atmosphere. Applicants disagree.

Again, the present invention is drawn to an improved method of preparing a nucleic acid array on a solid support. The synthesis steps are carried out in a non-oxidizing atmosphere. Applicants have provided comparative results that illustrate the decrease in signal obtained for oligonucleotides prepared by methods wherein oxidizing agents such as ozone are present. Indeed, Figure 1B of the instant application shows the decrease in probe yield obtained for hybrization of a mixed 16-mer probe, exposed to

ozone. As the Figure indicates, the mixed 16-mer is still present, even after 15 hours of exposure. Accordingly, the mixed 16-mer can still bind in a hybridization assay, but the signal produced will be significantly less than that of a probe array prepared according to the present methods.

Further, the Examiner concludes,

> It appears that the solutions used by Fodor et al. did not contain levels of ozone (e.g. 5 ppb) sufficient to cause degradation of poly dT, anticipating present claim 3. (Paper No. 5, *ibid.*)

Again, the observation that probes prepared by a known method work for their intended purpose, is not a valid anticipation of what is presented in the instant application as an improvement.

For the reasons provided above with regard to Terrett, Applicants respectfully request that this rejection be withdrawn.

Rejection of Claims 1, 2, 3 and 7-15 Under 35 USC § 103(a) ("Terrett")

Claims 1, 2, 3 and 7-15 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Terrett.

The Examiner acknowledges that Terrett fails to explicitly teach a "non-oxidizing atmosphere" and is silent with respect to ozone concentrations in the solutions used during synthesis, storage or screening.

However, the Examiner then provides the erroneous conclusion that the methods used for producing arrays that were fully functional in binding assays, indicates that ozone concentrations did not adversely affect the libraries (see Paper No. 5, page 8 first full paragraph) and further concludes that the concentration of ozone must have been in the range of about 0 to about 5 ppb.

Again, the rationale presented by the Examiner fails to consider that fully functional libraries or arrays can be prepared by methods other than the present claimed methods.

Terrett fails to disclose or *suggest* that ozone levels of from less than 5 ppb would provide improved arrays that are produced by the presently claimed methods. , and that the quality of the array prepared according to Applicants' methods is improved.

In the absence of some recognition that decreasing ozone levels in the atmosphere would lead to the improved products provided by the presently claimed methods, Applicants submit that a prima facie case of obviousness has not been set forth by the Office and respectfully request that this rejection be withdrawn.

Rejection of Claims 1, 2, 3 and 7-15 Under 35 USC § 103(a) ("Terrett and Fodor")

Claims 1, 2, 3 and 7-15 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Terrett and Fodor.

For the reasons provided above with regard to each of Terrett and Fodor, and in the absence of some motivation, other than hindsight recognition, that decreasing ozone levels in the atmosphere would lead to the improved products provided by the presently claimed methods, Applicants submit that a prima facie case of obviousness has not been set forth by the Office and respectfully request that this rejection be withdrawn.

Rejection of Claims 4-6 Under 35 USC § 103(a) ("Terrett and Fodor and Urdea")

Claims 4-6 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Terrett and Fodor, et al., and Urdea, U.S. Patent No. 4,517,338. Applicants respectfully traverse.

Terrett and Fodor et al. have been discussed above.

Urdea, et al. U.S. Patent No. 4,517,338 describes a multiple reactor system for polynucleotide synthesis in which reagents are moved throughout the system by the means of inert gases. The Examiner notes that it would have been obvious to conduct automated nucleic acid synthesis in the presence of an inert gas such as argon, nitrogen, helium or carbon-filtered air to protect the reagents from reacting with water. However,

Table 1, columns 9 and 10 of Urdea describe the use of several reagents with aqueous solvent systems. In particular, oxidation steps are carried out with iodine in the presence of lutidine/THF/H_2O (step 12) with steps 11 and 13 also utilizing water.

Turning next to particular limitations in the present claims, Applicants respectfully note that Claim 1, upon which Claims 4-6 depend, recites methods carried out in a non-oxidizing atmosphere having less than 5 ppb ozone. A complete reading of Urdea fails to turn up a single mention of nucleic acid synthesis wherein an atmosphere having ozone levels of less than 5 ppb is used. In fact, the Urdea reference merely describes a system wherein reagents are moved around by the use of gases.

In the absence of some motivation or suggestion to conduct synthesis under an atmosphere that has less than 5 ppb ozone, Applicants submit that a prima facie case of obviousness has not been set forth. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Rejection of Claims 4-6 Under 35 USC § 103(a) ("Terrett and Fodor and Brennan")

Claims 4-6 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Terrett and Fodor, et al., and Brennan, U.S. Patent No. 5,814,700. Applicants respectfully traverse.

Terrett and Fodor et al. have been discussed above.

Brennan, U.S. Patent No. 5,814,700 describes a method for preparing oligonucleotides wherein the reagents are moved via the use of an inert gas. The Examiner also notes that Brennan discloses at column 8 lines 40-55 that water and oxygen should be excluded from the reaction chamber in which phosphoramidites are used because phosphoramidites are sensitive to hydrolysis by traces of water and to oxidation by contact with air.

Applicants' invention goes significantly beyond the mere avoidance of water and ambient air during phosphoramidite coupling reactions. More particularly, claim 1 recites that "the synthesis steps are carried out in a non-oxidizing atmosphere"

having less than 5 ppb ozone. The synthesis steps include deprotection steps, coupling steps, and further deprotection steps. In some embodiments, packaging of the oligonucleotide arrays is conducted in a facility that is controlled to minimize exposure to oxidants such as ozone (see claim 12). According to Brennan, only the phosphoramidite reagents are sensitive to water or air. As a result Brennan fails to provide that which is missing from the prima facie case allegedly set forth by the Examiner, namely motivation to carry out all synthesis steps in an atmosphere that is non-oxidizing, and in particular, has an ozone concentration of less than 5 ppb.

Still further, in each of Urdea (cited above) and Brennan, the Examiner notes that inert gases are used to move reagents in a system to minimize exposure to air and water. However, neither of these references disclose or suggest that ozone should be excluded or minimized from the atmosphere. In fact, a word search of each of U.S. Patent Nos. 4,517,338 (Urdea), 5,424,186 (Fodor), and 5,814,700 (Brennan) conducted on the USPTO database, failed to turn up a single mention of ozone.

In the absence of some suggestion or motivation, other than hindsight recognition, that decreasing ozone levels in the atmosphere would lead to the improved products provided by the presently claimed methods, Applicants submit that a prima facie case of obviousness has not been set forth by the Office and respectfully request that this rejection be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5015

Respectfully submitted,

William B. Kezer
Reg. No. 37,369

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
WBK:jkh
WC 9037945 v3

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims

Claim 14 has been canceled.

Claims 1, 3, 7, 8, 9, 11 and 13 have been amended as follows:

1. (Amended) A method of preparing a nucleic acid array on a support, said method comprising synthesizing a plurality of nucleic acids on said support wherein the synthesis steps are carried out in a non-oxidizing atmosphere having less than 5 ppb ozone, and wherein said non-oxidizing atmosphere is an atmosphere that produces less than 10% degradation of said nucleic acids over a period of 1 hour at a temperature of from 18°C to 25°C.

3. (Amended) A method in accordance with claim **1**, wherein said synthesizing comprises the sequential steps of:

a) generating a pattern of light and dark areas by selectively irradiating at least a first area of a surface of a substrate, said surface comprising immobilized nucleotides on said surface, said nucleotides capped with a photoremovable protective group, without irradiating at least a second area of said surface, to remove said protective group from said nucleotides in said first area;

b) simultaneously contacting said first area and said second area of said surface with a first nucleotide to couple said first nucleotide to said immobilized nucleotides in said first area, and not in said second area, said first nucleotide capped with said photoremovable protective group;

c) generating another pattern of light and dark areas by selectively irradiating with light at least a part of said first area of said surface and at least a part of said second area to remove said protective group in said at least a part of said first area

and said at least a part of said second area;

d) simultaneously contacting said first area and said second area of said surface with a second nucleotide to couple said second nucleotide to said immobilized nucleotides in at least a part of said first area and at least a part of said second area;

e) performing additional irradiating and nucleotide contacting and coupling steps so that a matrix array of at least 100 nucleic acids having different sequences is formed on said support;

with the proviso that steps (a) through (e) are performed in said non-oxidizing atmosphere, and said atmosphere has an ozone concentration of less than [from about 0 to about] 5 ppb.

7. (Amended) A method in accordance with claim **3**, wherein said substrate is a planar support and is irradiated with light directed from a source at a position opposite the surface comprising said immobilzed nucleotides.

8. (Amended) A method in accordance with claim **3**, wherein said substrate is a planar support and is irradiated with light directed from a source on the same side of the surface comprising said immobilized nucleotides.

9. (Amended) A method in accordance with claim **3**, wherein said substrate is a planar support and is irradiated with light from a position opposite the surface comprising said immobilzed nucleotides and said atmosphere is an inert gas atmosphere.

11. (Amended) A method in accordance with claim **10**, wherein each of said steps is conducted in [a facility having] an atmosphere comprising less than 5 ppb [or less] ozone.

13. (Amended) A method of preparing a nucleic acid array, said method comprising attaching ~~each of a plurality of nucleic acids~~ to a solid support at preselected locations to provide said array, wherein said attaching is carried out in a non-oxidizing atmosphere having less than 5 ppb ozone, and wherein said non-oxidizing atmosphere produces less than 10% degradation over a period of 1 hour at a temperature of from 18°C to 25°C.